Study code: 3000-4202
(Old code 1903002)

Study title:

Effects on duration of pentobarbital-induced sleeping time in rats. Hydroxymatairesinol.

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Study Report

EFFECTS ON DURATION OF PENTOBARBITAL-INDUCED SLEEPING TIME IN RATS

HYDROXYMATAIRESINOL

Study number: **P11.4-1999**

Date: 20.8.2002 (version 2)

Sponsor

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Sponsor Study number: 1903002

CONFIDENTIAL

PreFa
Preclinical Pharmacology Research Unit
University of Turku

Key Words: Hydroxymatairesinol (HMR), safety pharmacology, barbiturate-induced sleeping time, drug interaction

1. GENERAL

1.1. SIGNATURES

Title Effects on duration of pentobarbital-induced sleeping in rats; Hydroxymatairesinol

PreFa study number: P11.4-1999

Sponsor study number: 1903002

Test item: Hydroxymatairesinol (HMR)

This Report version 2 replaces the 1st version dated 20.4.2000. Following changes have been made:

1. **Section 2.3.4. Rationale for dose selection:** Reference to a study demonstrating the antitumor activity of HMR has been added.
2. **Section 5.1, line 6:** misprint has been corrected: HTS-101 has been replaced with HMR.
3. **Table 5.1:** mistakes in descriptive statistics about the pentobarbital-induced sleeping time in groups treated with HMR have been corrected

This report is a complete and accurate account of the methods employed and the data obtained

20.8.2002

Aapo Honkanen
Study Director

date

1.2. TABLE CONTENTS

1.3. OBJECTIVE / PURPOSE OF THE STUDY

The purpose of this study was to assess general and safety pharmacological properties of the compound hydroxymatairesinol (HMR) by assessing its effect on pentobarbital-induced sleeping time. In addition to HMR, the effects of another compound, HTS-101 were tested in the same experiment. Same control group (vehicle treatment) and reference compound-treated group were used in the evaluation of the effects of these compounds. The results from HTS-101 are reported separately.

1.4. SUMMARY

Vehicle (PEG 300), different doses of hydroxymatairesinol (10, 30, 100 mg/kg, p.o.) were given 90 min before pentobarbital injection (40 mg/kg, i.p., 1 ml/kg). The reference compound, medetomidine, (0.05 mg/kg, s.c.), was given s.c. 15 min before pentobarbital. Pentobarbital-induced sleeping time was studied by measuring the length of loss of righting reflex.
Only medetodine significantly extended the pentobarbital induced sleeping time (vehicle group: 80 ± 8 min, mean ± S.E.M. and medetomidine group: 177 ± 3 min) all doses of test compound being without significant effect. One animal preatreated with 100 mg/kg of HMR died after pentobarbital treatment. These results suggest that hydroxymatairesinol do not potentiated central nervous system depressing effect of pentobarbital.

1.5. GUIDELINES

The study procedures described were based on the guidelines listed below:

- Asetus Kokeellisiin ja muihin tieteellisiin tarkoituksiin käytettävien selkärankaisten eläinten suojelemiseksi tehdyn eurooppalaisen yleissopimuksen voimaansaattamisesta. Suomen säädöskokoelma n:o 1360/90. Helsinki, 21 joulukuuta 1990
- European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes, European Treaty Series No. 123, (EU n:o 609/86) (Official Journal of the European Communities No L 358) Strasbourg 24th November 1986.

1.6. APPROVAL FROM THE ANIMAL CARE AND USE COMMITTEE

The study has a permission from the animal care and use committee of University of Turku n:o 922/99.

1.7. SPONSOR

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1.9. STUDY DIRECTOR

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1.10. PERSONNEL INVOLVED IN THE STUDY

PreFa/Department of Pharmacology and Clinical Pharmacology
Esa Korpi, Professor of Pharmacology
Aapo Honkanen, Study Director
Elisa Riuttala, Laboratory Technician

CRST/Biostatistics
Esa Wallius

1.11. TIME TABLE

Start of animal acclimatisation:	1.9.1999
Experimental starting date:	13.9.1999
Experimental completion date:	5.10.1999

2. MATERIALS AND METHODS

2.1. TEST SYSTEM/SUBJECTS

Experimental animals:	Sprague-Dawley Hsd:SD
Weight:	338 ± 19 g (Mean ± S.D.)
Source:	Harlan Winkelman GmbH, Germany
Number of animals in the study:	40
Number of animals/group:	8
Acclimatisation period:	13 days before start of the experiment.
Principles for selection into test groups:	Animals were selected randomly by hand into various test groups.
Identification of animals:	The animals were marked on their tails with with numbers in different colours.
Grounds for selection of species:	Rats are commonly used in studies of this type.

2.2. ENVIRONMENTAL CONDITIONS

Animal care:	The animals were cared and checked daily by the experimenters and/or personnel of the Central Animal Laboratory. The bedding of the animals was changed twice and water bottles once a week.
Number of animals/cage:	3 rats/cage.
Cage Type:	Polycarbonate Macrolon III (Scanbur AS, Denmark).
Bedding:	Aspen chips (Tapvei Oy Kaavi, Finland). The results of the analysis for specified contaminants are attached (Appendix 3).
Water:	Community tap water, *ad libitum*, except during the experiments. The results of the analysis for specified contaminants are attached (Appendix 4).
Fodder:	RM1 (E) SQC, Special Diet Service, Witham Essex, England, except as specified in the description of individual

experiments. Certificate detailing nutritional composition and levels of specified contaminants is attached (Appendix 5).

Ambient temperature:	21 ± 2.5 °C
Humidity:	50 % ± 15 %
Illumination:	12-h dark/light cycle; lights on from 7.00 to 19.00 and lights off from 19.00 to 7.00.
Room numbers:	Experimental room: 313 Colony room: 309

2.3. REAGENTS

2.3.1. Test compound

Hydroxymatairesinol (HMR) (mw. 374)

Vehicle:	PEG 300 Sigma (Chemicals Co, St Louis, MO, USA)
Batch:	00799
Storage:	at 4 °C, desiccated, protected from direct light

2.3.2. Reference compound

Medetomidine (mw. 200.28, Domitor® 1 mg/ml)

Manufacturer:	Orion Pharma (Espoo, Finland)
Vehicle:	0.9 % NaCl (saline)
Lot:	ZH 31-3
Batch:	11/98
Storage:	at room temperature protected from direct light

2.3.3. Other reagents

Pentobarbital (mw. 248.26, Mebunat® 60 mg/ml Orion Pharma)

Vehicle:	0.9 % NaCl
Lot:	VI 1-1
Batch:	07/98
Sorage:	at room temperature protected from direct light

2.3.4. Rationale for dose selection

In the experiments assessing the pharmacodynamic efficacy of HMR ,e.g. antitumor activity (Saarinen et al. Nutrition and cancer 2000 (36):207-216) a dose 15 mg/kg, (p.o.) have been found to be effective.

Thus, the doses selected for the present study (10, 30 and 100 mg/kg, p.o.) were within this therapeutic range or exceeded that.

2.3.5. Preparation and handling of test compound solutions

Fresh test compound solutions were prepared on each experimental day. HMR was dissolved in Polyethylene glycol (PEG) 300. Reference compound medetomidine was diluted from Domitor solution with 0.9 % NaCl. Test compound solutions were sonicated at 40 °C for 8-15 min. Pentobarbital solution used in the experiment was also prepared daily. Medetomidine test solution was prepared once a week.

2.4. EXPERIMENT

2.4.1. Procedure

The animals were habituated to handling and oral administration before the start of testing. The food but not water was withdrawn 18 h before the start of the experiment, and animals were transferred to cages with grid floors.

On the experimental day, the beginning of pentobarbital-induced sleeping was defined as the moment when the rat remained on its back when placed in this position (loss of righting reflex). The end-point of sleeping was defined as the moment when the animal spontaneously turned onto its feet and got up when the experimenter put it on its back again.

2.4.2. Administration of compounds

Vehicle (PEG 300), different doses of HMR were given p.o. (2 ml/kg) 90 min before pentobarbital injection (i.p., 1 ml/kg). Medetomidine, the reference compound, was given s.c. 15 min before pentobarbital (1 ml/kg).

Treatments

Groups	Treatment I	Treatment II
I	Vehicle (PEG 300)	Pentobarbital 40 mg/kg
II	Medetomidine, 0.05 mg/kg	Pentobarbital 40 mg/kg
III	HMR, 10 mg/kg	Pentobarbital 40 mg/kg
IV	HMR, 30 mg/kg	Pentobarbital 40 mg/kg
V	HMR, 100 mg/kg	Pentobarbital 40 mg/kg

n, = 8, n = 40

Data collection

Time elapsed from the pentobarbital injection until the loss of righting reflex and until the reappearance of righting reflex was recorded and entered manually into the worksheet.

Statistics

Means, standard deviations and standard errors for each group were calculated. The data were tested with analysis of variance (ANOVA) and between-group comparisons were made with Tukey post-hoc test.

2.4.3. Termination of the experiments

At the end of the experiment, all surviving animals were sacrificed with CO_2.

3. ARCHIVING

Study plan, final report and original data from different experiments are retained in the archive of PreFa (Tykistökatu 6B) for 10 years following approval of final report. After that, the further treatment of the documentation is decided together with the Sponsor. The documentation or parts of it may be delivered to the Sponsor on request before 10-year term. No data or documentation will be destroyed without permission from the Sponsor.

4. DEVIATIONS FROM STUDY PLAN

The experiment was performed as described in the Study Plan.

5. RESULTS

5.1. EFFECTS ON PENTOBARBITAL-INDUCED SLEEPING TIME

Body weights and pentobarbital-induced sleeping times of the animals in the different treatment groups are shown in Table 5.1. There were no differences in the body weights of the animals between the groups ($F = 0.60$, $p = 0.66$, ANOVA). Effects of different treatments on pentobarbital-induced sleeping time differed significantly ($F = 28$, $p < 0.0001$). However, only medetodine-treated group differed significantly from the control group ($p < 0.0001$) and from all HMR-treated groups (all $p < 0.0001$) in post analysis (Tukey's test). One animals pretreated with 100 mg/kg of HMR died 41 min after pentobarbital injection after showing irregular breathing.

Table 5.1. Average body weights and pentobarbital-induced sleeping times of animals in different treatment groups.

	Body Weight (g)			Sleeping time (min)				
Treatment	**Mean**	**S.D.**	**Range**	**Mean**	**S.D.**	**S.E.M.**	**Range**	**n_i**
Vehicle	343	29	288-374	80	24	8	46-121	8
Medetomidine	343	14	320-355	177	10	3	166-189	8
HMR 10	333	18	312-372	79	17	6	49-107	8
HMR 30	333	15	316-358	89	32	11	41-142	8
HMR 100	340	17	313-366	76	25	9	43-115	7

6. DISCUSSION AND CONCLUSIONS

The results indicate that HMR does not potentiate the central nervous system depressing effects of pentobarbital with the doses used. The reason for death of one HMR-treated animal during experiment is not known. Given the narrow therapeutic window of pentobarbital, it is possible that it was due to anaesthesia and not the test compound.

7. DISTRIBUTION OF THE REPORT

The Report is written in duplicate, one original copy being retained in the Archives of PreFa and one delivered to the Sponsor.

Appendices

1. Values from the individual animals
2. Statistics
3. Report from analysis of bedding for contaminants
4. Report from analysis of water for contaminants
5. Report from analysis of fodder for nutritional composition and levels of specified contaminants.